Chapter

Adaptive Drug Resistance in Malaria Parasite: A Threat to Malaria Elimination Agenda?

Moses Okpeku

Abstract

Malaria is a global disease of importance, especially in the sub-Saharan African region, where malaria accounts for great losses economically and to life. Fight to eliminate this disease has resulted in reduced disease burden in many places where the diseases is endemic. Elimination strategies in most places is focus on the use of treated nets and drug application. Exposure of malaria parasites to anti-malaria drugs have led to the evolution of drug resistance in both parasites and host. Development of drug resistance vary but, studies on adaptive drug resistance has implications and consequences. Our knowledge of this consequences are limited but important for the pursuit of an uninterrupted malaria elimination agenda. This chapter draws our attention to this risks and recommends interventions.

Keywords: adaptive resistance, drug-resistance, malaria, plasmodium, parasite

1. Introduction

1.1 Malaria - a global infectious disease

Malaria is a global deadly communicable disease [1], caused by plasmodium species, an apicomplexan microbe transmitted by the mosquito vector. Five major plasmodia parasites (*P falciparum, P vivax, P ovale, P malariae* and *P knowlesi*) have been implicated in malaria infections [2]. Of these, *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax (P. vivax)* are more widely distributed [3]. In sub-Saharan Africa, *P. falciparum* is the cause of most malaria cases while *P. vivax* is reported to cause most of the malaria cases in Asia; *P. falciparum* cause more fatal disease [4].

In 2019 alone, about 229 million positive malaria infections were reported globally, mortality from was estimated at about 409,000, with children under 5 years accounting for about 67% of death [5]. The disease is common among in poor communities [6], especially rural communities of the underdeveloped/developing countries of the world. Economic, social and health importance of this disease in terms of loss of life (particularly; young children), reduction in productivity of affected adult population, and negative social and health implication of the disease makes it one of the high ranking microbial, infectious disease in the world.

1.2 Malaria elimination agenda

Among the diseases which have great public health impact, malaria is a significant public health concern [7–9]. The fight to eliminate malaria is an aged long battle globally. Elimination programmes were launched after the Second World War [6], with chloroquine as the major frontline anti-malaria drug [10] and Dichlorodiphenyltrichloroethane DDT used for vector control [11]. Malaria eliminations efforts in Africa began with the World Health Organisation (WHO) roll back malaria initiatives started in 1998 [1]; these efforts focused on entomology control (to reduce transmitting vectors) using indoor insecticides, and treated mosquito nets introduced for protection and prevention [6].

The success of these elimination programmes is why over one hundred countries have been awarded malaria free status [12] and thirty-four others accorded elimination status [13], and most malaria endemic countries working very hard towards the attainment of elimination status. Today the disease burden continues to decrease across the world [14] relative to the era, before the launch of the global elimination programmes.

Success stories leading to this elimination stage in malaria control had been heavily dependent on traditional entomology surveillance and drug use. However, the plasmodium is a ubiquitous parasites that has evolved, complex systems of survival [15]. Among these survival strategies is the development of drug resistance to nearly all know malaria drugs. Resistance to chloroquine (the major frontline medicine for the treatment of malaria) was reported and widespread [13] long before the roll-back-malaria initiatives. Better understanding of the biology of the parasites and the life cycle led to the development of a range of other anti-malaria drugs some of which are still actively being used, but resistance to almost all know malaria drugs have been reported [16–19].

Drug resistance vary, and is transferable from pathogen to host [20]. Of the different types of drug resistance, adaptive drug resistance is usually not permanent, but is capable of producing strains of parasites not targeted by known drugs. This chapter aims at reviewing the different types of drug resistance with focus on adaptive drug resistance in plasmodium and the implication to malaria elimination programme.

2. Brief history of anti-malaria drug resistance

The fight against drug resistance in pathogenic microbes is global. The life of these microbes are so inter-twined with human wellness that if overlooked could be very costly in terms of treatment cost and loss of life. As efforts are being up-scaled towards malaria elimination, the issue of drug resistance continues to surface as a major challenge to cope with. This is because the malaria parasite continues to evolve and regularly develop mechanisms for surviving the toxic effect of drugs. These mechanisms result with fixed mutations in the genetic architecture that confers fitness and resistance to withstand or evade targeting drugs, thereby hindering or completely preventing binding between drug compounds and their target.

The history of evolution of drug resistance in plasmodium dates back to the 1930s when chloroquine (CQ) resistance in *P. falciparum* arose independently in Columbia and Thailand [21], and rapidly spread throughout the world. Research efforts to truncate this spread led to the development of different variants of malaria drugs to replace CQ. However, the plasmodium in it's unique way continue to adapt and evolve new mutations for survival and resistance to drugs which are harmful to it [22]. Advances in molecular technology has made it possible to

Mutation sites associated with drug resistance in plasmodium	References
Mutation resulting in polymorphism at the position 76 (K76T) in the transmembrane protein, known as *Plasmodium falciparum* chloroquine resistance transporter (PfCRT).	[23, 24]
The N86Y and Y184F amino-terminal mutations falciparum multidrug resistance transporter 1 (PfMDR1) has been implicated commonly in CQ and other anti-malaria drug resistance in Asian and African parasites.	[17, 25, 26]
Mutation of dihydrofolate reductase (DHFR) associated with *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance.	[27]
Mutation of dihydropteroate synthetase (DHPS) enzymes implicated in *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance.	[27]
mutations in *pvmdr1*, *pvcrt-o*, *pvdhfr*, and *pvdhps* genes in temperate-zone of *P. vivax associated with malaria drug resistance*	[28, 29]

Table 1.
Common mutations associated with P. falciparum *malaria drug resistance.*

uncover different mutations in the plasmodium parasites associated with drug resistance (**Table 1**). Evolution of these mutations are dynamic and difficult could be difficult to track and eliminate, especially when novel parasite results, against which known anti-malaria drugs is ineffective.

3. Drug resistance types: how much do we know in plasmodium?

Drug resistance types include Intrinsic, acquired and adaptive resistance. Intrinsic drug resistance is a natural phenomenon, and an innate ability in pathogen for resisting drug or harmful substance without prior record of susceptibility [30, 31], pathogens do not necessarily develop mutation for this to occur [32]. Acquired drug resistance builds up in human host, and makes them unresponsive to a drug that should normally eliminate known pathogenic parasite from the host system [33], these are both stable forms of drug resistance. Adaptive resistance [34, 35] develops in a pathogen in response to stimuli [36].

While "intrinsic and acquired resistance are stable and can be transmitted vertically to subsequent generations "[32] adaptive resistance is temporal, unstable, and is often lost ([17]; [37]). [38] observed that "unstable adaptation contains modulation of gene expression, which results in phenotypic changes due to changes in environmental markers that are sensed by the microorganisms" but it is not certain how long this resistance is, or could be sustained [39]. Adaptive resistance is acquired through mutation and binding genetic plasticity that enables transfer of genes [20] from parasites to host. These different mode of drug resistance have been extensively studied and reported for bacteria [36, 40–42], but not much is seen in literature regarding adaptive resistance in plasmodium.

4. Adaptive drug resistance has implications and consequences

Development of drug resistance interferes with disease control, increase the cost of treatment and management of control programs and if not quickly address could thwart control programmes. The evolution of drug resistance in malaria parasites have been a focus of many research but there is a dearth of information regarding adaptive resistance in malaria parasite and the consequence in their human hosts. It is quite understandable since adaptive resistance only confers a temporal resistance

and is reversible. Although temporal and reversible, The possibility of mutation and evolution of a unique strain of parasite is possible, on which known drugs would be ineffective. However, the period between active activation of adaptive resistance in plasmodium, the product of activation (whether lethal or not, or novel and insensitive to known drugs or not), the consequence in gene transfer to host and a host of other factors are unknown.

5. Discovering and tackling adaptive drug resistance in plasmodium: recommendations

Evolution of resistance to drugs is a survival mechanism influenced by many factors that produce mutation in the parasites. Common among causes of resistance is exposure to non-lethal doses of anti-malaria drugs [15]. Malaria parasites have unique ability to evolving mechanisms for evading the immune response in humans [43] and they are actively evolving resistance to anti-plasmodia drugs [44]. But there is a dearth of information are to the effect of plasmodia resistance to drug, especially adaptive resistance, which though is temporal, could influence the development of novel plasmodium stains not targeted by currently available anti-malaria drug. This development is a threat to malaria elimination agenda and should not be encouraged.

A host of resistance gene markers in plasmodium for drug resistance is an active field of malaria research [16, 18, 19, 45, 46], and still counting, but not much is written about the role or influence of adaptive resistance on these markers this is a conspicuous research gap in malaria biology and genetics requiring urgent attention. Selective sweep resulting in sudden change in an advantageous gene under strong positive selection [47] has been reported as product of evolution of resistance to drug. It is possible to scanning the genome for signature of selective sweeps, to identify genes undergoing adaptive evolution [48]. Similar studies revealed the mutations in presently known markers used in the study of malaria drug resistance [49–52], but none is focused on adaptive resistance. This kind of studies leverage of the NEXT GENERATION sequencing technology which is very limited and still very expensive in developing countries, especially in countries with no direct funding of research by government, where malaria is endemic.

6. Pertinent questions and suggestions for the way forward

Is adaptive resistance in malaria parasites a challenge? Does it have a significant influence on combating and elimination of malaria particularly in malaria endemic regions in Africa? Understanding the effects of adaptive malaria drug resistance, in plasmodium, the vector and the human host will greatly contribute to malaria elimination agenda and reposition the malaria elimination programmes across the world with focus on sub-Saharan Africa as the hub. In addition, different populations respond differently to the same drugs. These differential responses are influenced by genetic variability in different ethnic groups within a population, which in turn can be associated with variation in resistance to given drugs. Identification of genes and gene pathways involved in adaptive resistance is also vital for developing markers for prediction and diagnosis and should be pursued.

Kim and Schneider [48] observed that, "by examining selective sweeps in many endemic areas with different demographic and epidemiologic characteristics" it would be possible to identify factors associated with adaptive resistance to malaria drugs and track epidemiological variables [53–56] for transmission

and development of treatment regimes, accurate drug prescription and be able to determine costs of resistance. Understanding the implication and consequences of adaptive resistance alongside other forms of drug resistances will play significant role in policy formulation and implementation for disease control, give vivid picture of how to manage malaria control and modelling of disease transmission.

Author details

Moses Okpeku
Discipline of Genetics, School of Life Sciences, University of Kwa-Zulu Natal, South Africa

*Address all correspondence to: okpekum@ukzn.ac.za

References

[1] World Health Organization 2015. Global Malaria Programme. Eliminating malaria. Geneva: World Health Organization. World Health. 2015;243.

[2] Talapko, J., Škrlec, I., Alebić, T., Jukić, M. and Včev, A. 2019. Malaria: The past and the present. Microorganisms, 7(6), 179. MDPI AG. http://dx.doi.org/10.3390/microorganisms7060179

[3] White, M.T., Karl, S., Koepfli, C. Longley, R.J., Hofmann, N.E., Wampflier, R., Felger, I., Smith, T., Nguitragool, W., Sattabongkot, J., Robinson, L. Ghani, A. and Mueller I. 2018. Plasmodium vivax and plasmodium falciparum infection dynamics: re-infections, recrudescences and relapses. Malar J 17 (170). https://doi.org/10.1186/s12936-018-2318-1

[4] de Jong, S.E., van Unen, V., Manurung, M.D. et al. Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity. Nat Immunol 22, 654-665 (2021). https://doi.org/10.1038/s41590-021-00911-7

[5] World Health Organization 2020. World Malaria Report. https://www.who.int/malaria/publications/world-repot-malaria-report-2019/en/. Accessed 10 September, 2020.

[6] Maharaj, R., Kissoon, S., Lakan V. and Kheswa, N. 2019. Rolling back malaria in Africa – Challenges and opportunities to winning the elimination battle. South African Medical Journal 2019;109(11b):53-56. DOI:10.7196/SAMJ.2019.v109i11b.14250

[7] Dewald, J.R., Fuller, D.O., Müller, G.C. et al. A novel method for mapping village-scale outdoor resting microhabitats of the primary African malaria vector, Anopheles gambiae . Malar J 15, 489 (2016). https://doi.org/10.1186/s12936-016-1534-9

[8] Gwitira, I., Murwira, A., Mberikunashe, J. et al. Spatial overlaps in the distribution of HIV/AIDS and malaria in Zimbabwe. BMC Infect Dis 18, 598 (2018). https://doi.org/10.1186/s12879-018-3513-y

[9] Shi, S. M., Shi, T. Q., Chen, S. B., Cui, Y. B., Kassegne, K., Okpeku, M., Chen, J. H., & Shen, H. M. (2021). Genome-Wide Scans for Ghanaian Plasmodium falciparum Genes Under Selection From Local and Chinese Host Populations. Frontiers in cellular and infection microbiology, 11, 630797. https://doi.org/10.3389/fcimb.2021.630797

[10] Sweeney, A.W. 2000. Wartime research on malaria chemotherapy. Parassitologia 42:33-46

[11] Russell, P.F. 1951. Some epidemiological aspects of malaria control with reference to DDT. *J Natl Malar Soc.*10:257-265

[12] WHO, (2019): Global Malaria Program - Countries and territories certified malaria-free by WHO https://www.who.int/teams/global-malaria-programme/elimination/countries-and-territories-certified-malaria-free-by-who (accessed February 2021)

[13] Cotter, C., Sturrock, HJW, Hsiang, MS., Liu, J., Phillips, AA., Hwang, J., Gueye, CS., Fullman, N., Gosling, RD & Feachem, RGA. 2013. The changing epidemiology of malaria elimination: new strategies for new challenges. The Lancet, 382(9895), 900-911, https://doi.org/10.1016/S0140-6736(13)60310-4.

[14] Nkumama I.N, O'Meara W.P, Osier F.H.A. Changes in malaria epidemiology in Africa and new challenges for elimination. Trends Parasitol. 2017;33:128-140.

[15] Fernández L, Breidenstein E.B, Hancock R.E 2011. Creeping baselines

and adaptive resistance to antibiotics. Drug Resist Updat 14: 1-21.

[16] Huang B, Deng C, Yang T, et al. 2015 Polymorphisms of the artemisinin resistant marker (K13) in plasmodium falciparum parasite populations of Grande Comore Island 10 years after artemisinin combination therapy. Parasites and Vectors. 8:1-8.

[17] Idowu, A.O., Oyibo, W.A., Bhattacharyya, S. Khubbar, M. Mendie, U.E., Bumah, V.V., Black, C., Igietseme, J. and Azenabor A.A. 2019. Rare mutations in *Pfmdr1* gene of *Plasmodium falciparum* detected in clinical isolates from patients treated with anti-malarial drug in Nigeria. Malar J 18, 319. https://doi.org/10.1186/s12936-019-2947-z

[18] Kamau E, Campino S, Amenga-Etego L, et al. 2015 K13-propeller polymorphisms in plasmodium falciparum parasites from sub-saharan Africa. J infect dis.211:1352-5.

[19] Oboh MA, Ndiaye D, Antony HA, et al. 2018. Status of Artemisinin Resistance in Malaria Parasite Plasmodium Falciparum from Molecular Analyses of the Kelch13 Gene in Southwestern Nigeria. Biomed Res Int.

[20] Dhawale A and Rath A 2014. Antibiotic resistance: A threat and challenge to society. Ann Appl Biosci 1: R1-R6.

[21] Zareen S, Rehman H.U, Gul N, et al. 2016. Malaria is still a life threatening disease review. *J Entomol Zool Stud JEZS*.105:105-12.

[22] Laxminarayan R. 2004 . Act now or later? Economics of malaria resistance. Am J Trop Med Hyg. 71(2 Suppl):187-195. PMID: 15331837.

[23] Johnson, D. J., Fidock, D. A., Mungthin, M., Lakshmanan, V., Sidhu, A. B., Bray, P. G., & Ward, S. A. 2004. Evidence for a central role for PfCRT in conferring plasmodium falciparum resistance to diverse antimalarial agents. Molecular cell, *15*(6), 867-877. https://doi.org/10.1016/j.molcel.2004.09.012

[24] Lakshmanan, V., Bray, P. G., Verdier-Pinard, D., Johnson, D. J., Horrocks, P., Muhle, R. A., Alakpa, G. E., Hughes, R. H., Ward, S. A., Krogstad, D. J., Sidhu, A. B., & Fidock, D. A. 2005. A critical role for PfCRT K76T in Plasmodium falciparum verapamil-reversible chloroquine resistance. *The EMBO journal*, *24*(13), 2294-2305. https://doi.org/10.1038/sj.emboj.7600681

[25] Calçada C, Silva M, Baptista V, Thathy V, Silva-Pedrosa R, Granja D, Ferreira PE, Gil JP, Fidock DA, Veiga MI. 2020. Expansion of a specific plasmodium falciparum PfMDR1 haplotype in Southeast Asia with increased substrate transport. *mBio* 11: e02093-20. doi:10.1128/mBio.02093-20.

[26] Veiga, M. I., Dhingra, S. K., Henrich, P. P., Straimer, J., Gnädig, N., Uhlemann, A. C., Martin, R. E., Lehane, A. M., & Fidock, D. A. (2016). Globally prevalent PfMDR1 mutations modulate plasmodium falciparum susceptibility to artemisinin-based combination therapies. Nature communications, *7*, 11553. https://doi.org/10.1038/ncomms11553

[27] Ahmed, A., Bararia, D., Vinayak, S., Yameen, M., Biswas, S., Dev, V., Kumar, A., Ansari, M. A., & Sharma, Y. D. 2004. Plasmodium falciparum isolates in India exhibit a progressive increase in mutations associated with sulfadoxine-pyrimethamine resistance. Antimicrobial agents and chemotherapy, *48*(3), 879-889. doi:10.1128/aac.48.3.879-889.2004

[28] Lu, F., Wang, B., Cao, J., Sattabongkot, J., Zhou, H., Zhu, G., Kim, K., Gao, Q., & Han, E. T. 2012.

Prevalence of drug resistance-associated gene mutations in plasmodium vivax in Central China. The Korean journal of parasitology, 50(4), 379-384. https://doi.org/10.3347/kjp.2012.50.4.379

[29] Zhao, Y., Wang, L., Soe, M.T. Aung, P.L, Wei, H., Liu, Z., Ma, T., Huang, Y., Menezes, L.J., Wang, Q., Kyaw, M.P., Nyunt, M.H., Cui, L. & Cao, Y. 2020. Molecular surveillance for drug resistance markers in *Plasmodium vivax* isolates from symptomatic and asymptomatic infections at the China–Myanmar border. Malar J 19, 281. https://doi.org/10.1186/s12936-020-03354-x

[30] Cox G, Wright GD. Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. Int J Med Microbiol. 2013;303(6-7):287-292. doi: 10.1016/j.ijmm.2013.02.009.

[31] Impey R.E., Hawkins D. A., Sutton J.M. and Soares da Costa T.P. 2020. Overcoming intrinsic and acquired resistance mechanisms associated with the Cell Wall of gram-negative bacteria. Antibiotics 9:(623), 1 – 19. doi:10.3390/antibiotics9090623.

[32] Rizi K.S, Ghazvini K, Noghondar M.K 2018. Adaptive antibiotic resistance: Overview and perspectives. J Infect Dis Ther 6: 363. doi:10.4172/2332-0877.1000363.

[33] Kempker R.R, Kipiani M, Mirtskhulava V, Tukvadze N, Magee M.J, Blumberg H.M. 2015. Acquired drug resistance in mycobacterium tuberculosis and poor outcomes among patients with multidrug-resistant tuberculosis. Emerg Infect Dis. 21(6):992-1001. doi:10.3201/eid2106.141873.

[34] Coleman SR, Bains M, Smith ML, Spicer V, Lao Y, Taylor PK, Mookherjee N, Hancock REW. 2021. The small RNAs PA2952.1 and PrrH as regulators of virulence, motility, and iron metabolism in *Pseudomonas aeruginosa*. Appl Environ Microbiol 87: e02182-e02120. doi:10.1128/AEM.02182-20.

[35] Coleman SR, Blimkie T, Falsafi R, Hancock R.E.W. 2020. Multidrug adaptive resistance of *Pseudomonas aeruginosa* swarming cells. Antimicrob Agents Chemother 64: e01999-e01919. doi:10.1128/AAC.01999-19.

[36] Fernández, L., & Hancock, R. E. 2012. Adaptive and mutational resistance: Role of porins and efflux pumps in drug resistance. Clinical microbiology reviews, 25(4), 661-681. https://doi.org/10.1128/CMR.00043-12

[37] Baquero F 2001. Low-level antibacterial resistance: A gateway to clinical resistance. Drug Resist Updat 4: 93-105.

[38] López-Maury L, Marguerat S, Bähler J 2008. Tuning gene expression to changing environments: From rapid responses to evolutionary adaptation. Nat Rev Genet 9: 583-593.

[39] Jahn L.J, Munck C, Ellabaan M.M, Sommer M.O .2017. Adaptive laboratoryevolution of antibiotic resistance using different selection regimes lead to similar phenotypes and genotypes. Front Microbiol 8: 816.

[40] Li, X. Z., Zhang, L., & Nikaido, H. 2004. Efflux pump-mediated intrinsic drug resistance in mycobacterium smegmatis. Antimicrobial agents and chemotherapy, 48(7), 2415-2423. https://doi.org/10.1128/AAC.48.7.2415-2423.2004

[41] Motta, S.S., Cluzel, P. & Aldana, M. 2015. Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps. PLoS ONE 10(3): e0118464. https://doi.org/10.1371/journal.pone.0118464

[42] Olaitan, A.O., Morand, S. & Rolain, J.M. 2014 Mechanisms of polymyxin

resistance: Acquired and intrinsic resistance in bacteria. Front. Microbiol. 5:643. doi: 10.3389/fmicb.2014.00643

[43] Dinko B, Pradel G. 2016. Immune evasion by plasmodium falciparum parasites converting a host protection mechanism for the parasite's benefit. Advances in Infectious Diseases.06(02):82-95.

[44] Niba, P.T.N., Nji, A.M., Evehe, MS. et al. 2020. Drug Resistance Markers within an Evolving Efficacy of Anti-Malarial Drugs in Cameroon: A Systematic Review and Meta-Analysis 1998–Malar J 20, 32 (2021). https://doi.org/10.1186/s12936-020-03543-8.

[45] Conrad MD, Bigira V, Kapisi J, et al. 2014. Polymorphisms in K13 and falcipain-2 associated with artemisinin resistance are not prevalent in Plasmodium falciparum isolated from Ugandan children. PLoS One. 9. 1 – 10.

[46] Ouattara A, Kone A, Adams M, et al. 2015. Polymorphisms in the K13-propeller gene in artemisinin-susceptible plasmodium falciparum parasites from Bougoula-Hameau and Bandiagara, Mali. Am J Trop Med Hyg. 92:1202-1206.

[47] Maynard, Smith J. & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. Genetical Research 23, 23-35.

[48] Kim, Y. & Schneider, K. A. (2013) Evolution of drug resistance in malaria parasite populations. Nature Education Knowledge 4(8):6

[49] Wootton JC, Feng X, Ferdig MT, Cooper RA, Mu J, Baruch DI, Magill AJ, Su XZ. Genetic diversity and chloroquine selective sweeps in Plasmodium falciparum. Nature. 2002 Jul 18;418(6895):320-323. doi: 10.1038/nature00813. PMID: 12124623.

[50] Nair, S., Williams, JT., Brockman, A., Paiphun, L., Mayxay, M., Newton, PN., Guthmann, J., Smithuis, FM., Hien, TT., White, NJ., Nosten, F. & Anderson, TJC. 2003. A Selective Sweep Driven by Pyrimethamine Treatment in Southeast Asian Malaria Parasites, Molecular Biology and Evolution. 20(9) 1526-1536, https://doi.org/10.1093/molbev/msg162

[51] Nash D, Nair S, Mayxay M, Newton PN, Guthmann JP, Nosten F, Anderson TJ. 2005. Selection strength and hitchhiking around two anti-malarial resistance genes. Proc Biol Sci. 272(1568):1153-61. doi: 10.1098/rspb.2004.3026. PMID: 16024377; PMCID: PMC1559806.

[52] Vinayak, Sumiti and Alam, Tauqeer and Mixson-Hayden, Tonya and McCollum, Andrea M. and Sem, Rithy and Shah, Naman K. and Lim, Pharath and Muth, Sinuon and Rogers, William O. and Fandeur, Thierry and Barnwell, John W. and Escalante, Ananias A. and Wongsrichanalai, Chansuda and Ariey, Frederick and Meshnick, Steven R. and Udhayakumar, Venkatachalam. 2010. Origin and Evolution of Sulfadoxine Resistant Plasmodium falciparum. PLOS Pathogens. 3. e1000830. Doi:10.1371/journal.ppat.1000830

[53] Belachew E.B. 2018. Immune response and evasion mechanisms of plasmodium falciparum parasites. J Immunol Res. 2018: 6529681.

[54] Escalante, A. A., Smith, D. L., & Kim, Y. (2009). The dynamics of mutations associated with anti-malarial drug resistance in Plasmodium falciparum. Trends in parasitology, 25(12), 557-563. https://doi.org/10.1016/j.pt.2009.09.008

[55] Schneider KA, Kim Y. An analytical model for genetic hitchhiking in the evolution of antimalarial drug resistance. Theor Popul Biol. 2010 Sep;78(2):93-108. doi: 10.1016/j.tpb.2010.06.005. Epub 2010 Jun 19.

PMID: 20600206; PMCID:
PMC2916054.

[56] Maharaj, L., Adeleke, VT., Fatoba, AJ., Adeniyi A A., Tshilwane, SI., Adeleke, MA., Maharaj, R. & Okpeku, M. 2021. Immunoinformatics approach for multi-epitope vaccine design against P. falciparum malaria. Infection, Genetics and Evolution. 92 104875. https://doi.org/10.1016/j.meegid.2021.104875.